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Preliminary Report

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Content

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Declarations of Interest

All participants completed a Declaration of Interests form in advance of their involvement in in this work.

One of the Experts declared an interest in the topic under consideration:

Dr Andrew Singer declared he owns stocks in various pharmaceutical and vaccine manufactures, which is over the threshold of FAO/WHO. Upon detailed review of the declaration, it was considered that the activities of Dr Andrew Singer represent a potential conflict of interest. Therefore, he was invited to the meeting, but did not participate in the final adoption of the conclusions and recommendations of the meeting.

All of the declarations, together with any updates, were made known and available to all the participants at the beginning of the meeting.

All the experts participated in their individual capacity and not as representatives of their country, government, or organizations.

Abbreviations

AMR	Antimicrobial resistance
AMU	Antimicrobial use
ARGs	Antimicrobial resistance genes
CAC	Codex Alimentarius Commission
ECOFFs	Epidemiological cut-off values
FAO	Food and Agriculture Organization of the United Nations
IPM	Integrated Pest Management
LMICs	Low- and middle-income countries
OIE	World Organisation for Animal Health
TFAMR	Ad hoc Codex Intergovernmental Task Force on Antimicrobial Resistance
WHO	World Health Organization

Executive summary

There is clear scientific evidence that foods of plant origin may serve as a vehicle of foodborne exposure to antimicrobial-resistant bacteria. Fruits, vegetables and other foods of plant origin can become contaminated with antimicrobial-resistant bacteria and antimicrobial resistance genes (ARGs) anywhere along the food chain, from primary production to consumption. Conventionally and organically grown vegetables to be consumed raw may be vehicles of dissemination of antimicrobial-resistant bacteria and their resistance genes to humans. Concerted efforts should be made to mitigate their contamination at all stages of the food chain, from production to consumption. Important sources of microbial contamination in the pre-harvest environment include soil, organic fertilisers and irrigation water. Hence, good agricultural hygienic practices should be employed during pre-harvest stages of food production.

Use of antimicrobials in humans and animals selects for antimicrobial-resistant bacteria in faeces. Up to 80% of the antimicrobial administered (as well as copper and zinc from the diet) is excreted in the faeces and urine in an active form. Thus, manure or other organic material that contain human or animal wastes used as soil amendments have the potential to disseminate both residues of antimicrobial agents and antimicrobial-resistant bacteria to the environment. Vegetables harvested from manured ground can carry an additional burden of ARGs of enteric or environmental bacterial origin.

Water can also be an important source of antimicrobial residues, antimicrobial-resistant bacteria and ARGs. There is a direct link between water quality used for irrigation and antimicrobial-resistant bacteria on foods. Wastewater effluent recovered from municipal sewage may contain ARGs and antimicrobial-resistant bacteria. Consequently, soils irrigated with wastewater can also become contaminated with ARGs and with multidrug antimicrobial-resistant bacteria. Water found adjacent to manured fields may also be enriched in antimicrobial-resistant bacteria.

Aquaculture products (*e.g.* fish, shellfish, and shrimp) can carry bacteria that are resistant to medically important antimicrobials. Aquaculture primary food production systems that receive antimicrobials, or that are exposed to effluents containing antimicrobial residues and/or faecal material of human or animal origin, can become enriched in antimicrobial-resistant bacteria. Additionally, aquaculture production has the potential to contaminate water used for irrigation. Using water contaminated with this effluent for irrigation purposes provides a direct route of contamination of fruits and vegetables, if such water is applied directly to the edible portions of the plant. Aquaculture systems can vary substantially between countries or regions in ways that may variably impact the risk of acquiring and disseminating antimicrobial resistance (AMR).

Special emphasis should be put on to so-called integrated food production systems. Here, crops are produced together with food of aquaculture origin, based on water contaminated with human or animal waste. This may constitute a resource efficient system, including from a waste management viewpoint. Chemical disinfectants are critical for food hygiene and environmental sanitation. Bacteria with increased tolerance to biocides have been recovered from food production environments. Although there is theoretical and experimental evidence that certain microbiocidal agents may co-select for AMR, there is an absence of empirical data to indicate that the use of biocides drives this co-selection under the conditions present in the food production or processing environments.

Antimicrobials are vital to treat and control plant diseases. Contamination of soils with these products following crop application leads to enrichment of antimicrobial-resistant bacteria and ARGs in the environment. However, the extent to which the treatment of crops with antimicrobial agents (or copper formulations, see below) promotes AMR in bacteria found on edible portions of fresh plant produce is uncertain.

Of concern is the possibility of selection of antimicrobial-resistant bacteria and ARGs through the processes of co-resistance, cross-resistance and co-regulation with certain metal ions. Contamination of soil with certain metal ions, such as copper ions, can promote AMR in soil bacteria. Not only are copper-containing products used to treat plant diseases, animal and human wastes often have residue levels of copper, zinc and other metals of dietary origin. Bacteria harbouring genes conferring resistance to certain metal ions (and in some cases to certain biocides) are more likely to also carry ARGs than those without such metal ion resistance traits. Bacteria resistant to both metal ions and antimicrobials are commonly present in diverse environments, with bacteria of plant origin having the highest frequency of resistance to both metals and antimicrobials, compared to bacteria from other sources such as domestic animals, wild animals or humans.

Given the potential of human exposure to antimicrobial-resistant bacteria via foods of plant origin and from aquaculture products, there is considerable value in incorporating these products into integrated antimicrobial use (AMU) and AMR surveillance systems. Although *E. coli* may serve as a suitable common indicator bacterium for antimicrobial-resistant bacteria in food of animal origin, there is a need to identify additional robust indicators of antimicrobial-resistant bacteria in foods of plant origin and the immediate crop production environment. Likewise, there are no universally accepted bacterial indicators of AMR in aquatic products. AMR surveillance should use culture and antimicrobial susceptibility testing based on epidemiological cut-off values (ECOFFs) and may need to include molecular methods for ARG analysis, and antimicrobial residue chemical analyses. Antimicrobial-resistant bacteria, ARGs and AMU surveillance in fruit and vegetable production systems should capture all important metadata for the antimicrobials such as information from manufacturers, importers, vendors, where possible.

Conclusions

- Best management practices should be adhered to with respect to the use of faecal material of human (sewage sludge; biosolids) or animal origin (manures) in primary food production environments. Likewise, the use of reclaimed wastewater for irrigation.
- Improved methods for infection prevention and control such as husbandry, biosecurity, diagnostics, vaccines and other alternatives and adjuvants to antimicrobials should be employed to reduce the need for AMU in aquaculture, and thereby reduce the antimicrobial contamination of the primary aquaculture production environment.
- Biocides should be used according to manufacturers' recommendations.
- Antimicrobials should only be used in crop production according to label guidelines in the context of integrated pest management strategies.

Introduction

In recognition of the growing problem of antimicrobial resistance (AMR), its increasing threat to human, animal and plant health, and the need for a One Health approach to address this issue, the 39th Session of the Codex Alimentarius Commission (CAC) agreed it was important for the food safety community to play its part and re-established the *ad hoc* Codex Intergovernmental Task Force on Antimicrobial Resistance (TFAMR)[1] with the objectives of the Task Force revising the current *Codex Code of Practice to Minimise and Contain Antimicrobial Resistance* (CAC/RCP 61-2005)[2] and to developing new guidance on surveillance programmes relevant to foodborne AMR.

Responding to the request from the CAC and the Task Force to provide scientific advice in the areas of crops, environment and biocides,[3] the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) convened, in collaboration with the World Organisation for Animal Health (OIE), a joint "FAO/WHO expert meeting on foodborne antimicrobial resistance: role of environment, crops and biocides" on 11-15 June 2018 in Rome, Italy.

The primary purpose of the meeting was to synthesize the current scientific literature concerning the transmission of antimicrobial-resistant bacteria, antimicrobial residues and antimicrobial resistance genes (ARGs) from environmental sources (*e.g.* contaminated water, soil, manure or human wastes, fertilizers, processing and transportation facilities) to foods and feeds of plant and aquatic animal origin. As a secondary goal, given the widespread and frequent use of disinfectants in food processing plant sanitation, the potential of biocides to co-select for AMR and ARGs was also reviewed. Non-food crops (*e.g.* cotton, flower bulbs) were excluded from this scope.

The meeting therefore addressed the following priority areas: the prevalence of antimicrobial-resistant bacteria and ARGs of fruit and vegetables (Section 1); antimicrobial residues, antimicrobial-resistant bacteria and ARGs in the immediate food production environment, namely in soils (Section 2.1), irrigation water (Section 2.2), aquaculture (Section 2.3), use of antimicrobials and copper in horticulture production (Section 2.4), and defining environmental quality thresholds for antimicrobials (Section 2.5); use of biocides in the food processing environment (Section 3); and crops, aquaculture products, and their production environments in integrated surveillance of AMR (Section 4).

Answering this request, FAO and WHO, in collaboration with OIE, have been planning a series of expert consultations to firstly address the risk assessment aspects and then address the risk management component of the request from the Codex Alimentarius Commission. Furthermore, Codex requested that the scientific advice should seek to identify any further issues and specific gaps in current scientific knowledge that need to be considered in the revision of existing Codex texts and/or development of

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[1] REP16/CAC
[2] CAC/RCP 61-2005
[3] REP18/AMR

new Codex texts. To ensure transparency, a public call for experts[4] and data[5] were published and disseminated globally. Responses were received and suitable experts selected under consideration of any declared interests by the experts.

[4] http://www.fao.org/fileadmin/user_upload/agns/pdf/Call_for_data_experts/EXPERTS_Foodborne_AMR.pdf or http://www.who.int/foodsafety/Call_for_experts_oct2017.pdf
[5] http://www.fao.org/fileadmin/user_upload/agns/pdf/Call_for_data_experts/DATA_Foodborne_AMR.pdf or http://www.who.int/foodsafety/DATA_Foodborne_AMR.pdf

1. Contamination of crops with antimicrobial-resistant bacteria

The microbiological contamination of foods of plant origin (*e.g.* fruits, vegetables, lettuce) that are consumed raw or undercooked is responsible for foodborne illnesses worldwide, including outbreaks of disease caused by antimicrobial-resistant bacteria. In addition to antimicrobial-resistant pathogens, produce at retail can also be contaminated with non-pathogenic bacteria that may carry resistance to medically important antimicrobials (Verraes *et al.*, 2013; Thanner *et al.*, 2016).

Bacteria and fungi cause significant plant disease and production losses worldwide, especially in low- and middle-income countries (LMICs). Climate change is predicted to exacerbate this problem and the use of antimicrobial agents is expected to rise concomitantly as older treatments become ineffective and are discontinued and as disease burden continues to climb. There is growing concern that antimicrobials are losing their effectiveness in all sectors, not only in horticulture, but also in veterinary and human medicine. Extensive use and misuse of antimicrobials drives the development and transmission of AMR, but it is unclear the extent to which AMU is driving the development of AMR specifically in plant pathogens, soil organisms, spoilage organisms, and non-pathogenic contaminants and zoonotic agents present on foods of plant origin.

Some of the same drugs that are used in human and veterinary medicine (*e.g.*, streptomycin, tetracyclines, triazoles) are also used to control plant diseases. Thus, resistance that develops in one sector can be transferred and clinically relevant across sectors and species. Through processes of co-selection and cross-resistance, resistance that develops to one antimicrobial may also render an organism resistant to several unrelated drugs and chemicals. Bacteria, notably zoonotic organisms with resistance to one or multiple antimicrobials, are found on fruits, vegetables, and other edible plants, as well as in soils. The frequency with which plant-origin resistant bacteria colonize the human gastrointestinal tract or serve as reservoirs of AMR genes in the gastrointestinal tract needs to be determined. There is convincing evidence that agricultural AMU is driving the emergence of antimicrobial-resistant fungi that are increasingly transmitted from the environment to humans.

Several antimicrobials are approved for use to specifically treat bacterial plant diseases in at least 20 countries. In countries where regulations and oversight of AMU are strong, the use of antimicrobials and their residues on foods of plant origin is minimal. However, in other countries, the quantity and types of antimicrobials being used for agronomic application are undocumented – a problem compounded by challenges of access to quality-assured antimicrobials, including a growing industry of fraudulent and substandard products. The consequences of AMU in plant production resulting in occupational exposure, food, and environmental contamination need to be assessed in order to develop science-based recommendations for mitigating the negative public health impacts of AMR.

As fruits and vegetables are frequently eaten raw or with minimal processing, it can be stated that fresh fruits and vegetables serve as a source of dietary exposure to antimicrobial-resistant bacteria and ARGs. Other reports also confirm the role that foods of plant origin play in the foodborne transmission of antimicrobial-resistant bacteria (Bezanson *et al.*, 2008; Boehme *et al.*, 2004; Hassan *et al.*, 2011; Raphael *et al.*, 2011; Rodríguez *et al.*, 2006; Ruimy *et al.*, 2010; Schwaiger *et al.*, 2011; Walia *et al.*, 2013). Therefore, reducing the contamination of foods and feeds of plant origin with antimicrobial-resistant bacteria will reduce human and animal exposure to antimicrobial-resistant bacteria and ARGs.

Resistant bacteria from multiple sources can contaminate foods of plant origin. The soil is replete with bacteria that harbour ARGs. Direct contact of edible portions of plants with soil and soil splash can contribute to food contamination. Animal and human wastes introduced intentionally as soil amendments or through animal intrusion provide another pathway for antimicrobial-resistant bacteria to contaminate foods of plant origin. Water used for irrigation may also be contaminated with antimicrobial-resistant organisms. Importantly, the adoption of good agricultural practices that limit total microbial contamination of foods of plant origin is a critical first step in reducing the introduction of antimicrobial-resistant organisms into the food chain.

Contributing to the problem of AMR is the fact that there are very few products to treat bacterial infections of plants. The risk of development of resistance in plant pathogen populations is widely understood in horticultural production systems: Among bacterial plant pathogens, resistance is reported for products commonly used to treat bacterial diseases (*e.g.*, streptomycin, tetracycline, kasugamycin, and copper). With regard to antifungals, resistance to triazole fungicides is also well documented and relatively common, although there is a great deal of variation in the frequency of resistance between regions and among pathogens.

There are production practices available that can minimize AMR risks by reducing the need for AMU. Biological control (introduction of organisms that provide direct antagonism, competition, hyper-parasitism, or induction of host plant resistance) and biorational products, such as plant extracts, can prevent and treat plant diseases. These products are considered to be lower risk to the environment and human health. However, biological and biorational products are generally far less effective than antimicrobials and their performance is inconsistent over time and across locations. As more is learned about phytobiome functions in food crop systems, more effective pre- and pro-biotic agents against plant pathogens may be developed to reduce the need for conventional antibacterial and antifungal agents.

By far the most effective approach to limit AMU in plant production is through the use of the well-established procedures of "Integrated Pest Management" (IPM) – a systems approach designed to minimize economic losses to crops, as well as to minimize risks to people and the environment through the use of pesticides. Key components of IPM for preventing and managing plant diseases are:

- **Accurate and timely diagnosis and monitoring**, which can also include disease modelling and predictive systems to optimize timing of plant protection product applications;
- **Use of disease resistant crop varieties**, including resistant rootstocks in both fruit and vegetable systems;
- **Exclusionary practices** (biosecurity) that prevent the introduction of pathogens into a crop, such as using pathogen-free true seed and vegetative planting material, clean irrigation water and sanitation practices that prevent the movement of pathogens from plant to plant and field to field;
- **Careful site selection and soil improvement** to maximize plant health and minimize environmental factors that favor pathogens;
- **Crop rotation** and other cultural practices to prevent pathogen build-up;
- **Use of biological and biorational products**; and
- **Judicious use of antimicrobials**, including those for the treatment and control of bacterial and fungal diseases.

While many growers in developed countries are aware of and practice disease management strategies, improved uptake of these specific practices, especially in LMICs, will help to reduce infection pressure and consequently the need for antimicrobials. IPM should continue to be emphasized in grower and gardener education programs in developed economies and should be widely encouraged through governmental and non-governmental programs in LMICs. The importance of IPM for slowing the development of AMR and promoting food security and human and animal health cannot be overstated.

Additional information, tools, and activities are urgently needed to better understand and mitigate the risks associated with AMR from agronomic sources, especially in LMICs. For example, advances in surveillance, good practices, awareness and strengthened government regulation and oversight for AMU and surveillance will contribute to a more effective One Health approach to combat AMR.

The largest barrier to understanding the role of plant-based agriculture in the holistic picture of AMR ecology is the lack of relevant data. The data of the dissemination of antimicrobial-resistant organisms from crops that are consumed raw impacting human health is sparse. Information is particularly lacking for LMICs. Systems to record AMU and antimicrobial-resistant organisms on fruits and vegetables at the national level are virtually absent. Surveillance systems for foods of plant origin should be developed in such a way that they can be integrated and harmonized with surveillance in other sectors, including AMR programmes in humans, animals, and foods of animal origin, to better assess risks and priority areas for intervention. In addition to AMR among plant pathogens, it is important to monitor animal, human, and zoonotic pathogens on plants as well as the resistome of other organisms in the plant production environment, which may also contribute resistance genes to the food chain. The creation of new, rapid, and inexpensive tests and tools to diagnose plant diseases and characterize the resistome of the plant production environment will help to establish more appropriate surveillance strategies and AMU guidelines. To this end there are challenges that need to be addressed in developing these surveillance programmes. One key challenge will be determining an appropriate standard denominator to characterize AMU (*e.g.,* kilograms of oxytetracycline used per tonne of dates or apples produced) so that trends within and across countries can be monitored in kind.

Few methods are available to reduce or eliminate bacteria or ARGs from fruits and vegetables that are consumed raw or with minimal processing. Therefore, prevention of contamination at all stages of production and processing is paramount to minimize the introduction of antimicrobial-resistant organisms into the food chain from plant-based foods. Development, validation, and application of additional contamination prevention strategies along the entire food chain could greatly reduce antimicrobial-resistant organisms and ARGs in foods of plant origin.

Due to the limited number of medicines available to effectively treat plant diseases, additional strategies to prevent, control and treat plant diseases need to be developed, especially interventions and products with systemic effects. Examples of valuable innovations may include the following:

- **Selective breeding** to decrease host plant susceptibility to diseases or to enable plants to degrade antimicrobials to reduce soil contamination;
- **Discovery of drugs with antifungal and/or antibacterial activity** with different modes of action not shared with drugs used in human medicine;
- Use of **effective biologicals** (probiotics, prebiotics, bacteriophages) **and biorational compounds** for disease control;
- **Exploitation of the microbiome and soil health** to control plant diseases; and
- More effective **integrated disease and pest management strategies**.

Additional information is specifically needed to quantify the relationship between the use of antimicrobials, other plant protection products, and other influences on the selection, transmission, and persistence of AMR among organisms on plants and in the surrounding food production environment.

A paradigm shift in plant-based food production practices and acceptance of these practices by producers is needed to reduce AMU. Awareness of the severity of the problem and adoption of sustainable solution pathways at all stages in the food chain is critical to slow the development of AMR and mitigate its negative consequences. For food producers, this means recognizing that AMR can contribute to production and economic losses at all scales of production. Resistance can also cause direct and serious health impacts on producers who apply antimicrobials, and their families and customers who consume products contaminated with antimicrobial residues and antimicrobial-resistant bacteria (Marshall and Levy, 2011). In some countries, buyers are demanding commodities produced with strong antimicrobial stewardship practices, such as treating only after a correct diagnosis, appropriate application and dosing, respecting pre-harvest intervals, and incorporating IPM practices. Understanding and incentives for producers to employ better practices remains an obstacle to effective management of AMR and this problem is further complicated by misinformation and availability of products on the market that are fraudulent, substandard, or otherwise without evidence of effectiveness.

1.1 Crops for human consumption

Human and animal waste is frequently used as a valued fertilizer in crop production. If excreta are not treated properly, they may carry antimicrobial-resistant pathogens (Marshall and Levy, 2011). If plant food products that are consumed raw or undercooked are contaminated through environmental sources, they will be collectively contaminated with a whole range of bacteria and resistance and virulence genes from human, animal and environmental origin. Other sources of contamination include water, air and soil

1.2 Crops for animal consumption

Terrestrial animals (*e.g.* ruminants) will forage on pasture or are fed grains and grass crops (*e.g.* silage or haylage). It is common practice in production animal farms worldwide that the animal manure is used as a fertilizer for the crops that are grown for forage of the animals. Wildlife, pest animals and insects commonly inhabit farm settings and are known to be able to disseminate bacteria to and from production animals. The forage and feed crops may be contaminated with animal waste, including from both husbandry animals and wildlife (Fenlon, 1985; Nightingale *et al.*, 2004). Crops grown for animal feeds may also be contaminated with bacteria from the soil (Heyndrickx, 2011), which commonly harbour AMR genes (Wright, 2010). If antimicrobials are administered to production animals while the animals are consuming crops contaminated with AMR bacteria, selection for this population in the animal gut might occur. This could be a route of introduction and amplification of ARGs of environmental origin into the food chain (Marshall and Levy, 2011; Witte, 2000) and should be taken into account when considering if the animal feed and agricultural soils should be a part of the surveillance. Also, if practices in the production animal farms are modified that affect the of the frequency of contamination of livestock feeds, new and emerging unpredictable issues might arise. . Such practices might be for example the use of recycled fertilizers that might harbour contaminants that are not present in manure. In addition to manure and feces, wildlife, insects and pest animals are suspected to be a significant contributor in disseminating AMR between production animals and the environment (*e.g.* Surette and Wright, 2017); however, understanding their role and the possible risks would require systematic investigations. For example, research from Norway indicated the prevalence of AMR in *E. coli* from red foxes was higher in areas with higher human population density and in areas close to the larger cities than in less populated areas (Norwegian Veterinary Institute, 2017).

2. Antimicrobial-resistant bacteria and ARGs in the immediate plant production environment

Fruits, vegetables and other foods of plant origin can become contaminated with antimicrobial-resistant bacteria and ARGs anywhere along the food chain, from primary production to consumption. Conventionally and organically grown vegetables to be consumed raw may be vehicles of dissemination of antimicrobial-resistant bacteria and their resistance genes to humans (Zarfel *et al.*, 2013). Important sources of microbial contamination in the pre-harvest environment include soil, organic fertilisers and irrigation water.

Antimicrobials are widely used for people, livestock, poultry, aquaculture, apiculture, pets, and plants, not only for treatment of infections, but also for disease control, prophylaxis, and, in some countries, for growth promotion in food-producing animals. Depending on the species treated and the particular drug used, the percentage of the dosage that is absorbed or metabolized by an individual animal or person, ranges from as little as 10% to over 80%, with the remainder excreted as active compounds through urine and faeces into the environment. Soils are contaminated by antimicrobials used for disease control in plant production, and by residues in manures and wastes applied as crop fertilizers. Waste streams from humans and animals treated with antimicrobials are also enriched with resistant microorganisms and ARGs.

Persistence dynamics of antimicrobial residues, ARGs, and the survival of antimicrobial-resistant organisms in the environment are complex. A number of biotic and abiotic factors including temperature, solar radiation exposure, pH, soil type, and microbial biodiversity influence how long residues remain in the environment, and at what rate bacteria proliferate, die off, exchange resistance genes, and are dispersed. Of growing concern are the effects that low concentrations of antimicrobials (lower than minimal inhibitory concentrations of antimicrobials) have as a selective force in AMR emergence. The cross-resistance and co-selection can further contribute to the multi-drug resistance problem. Thus, resistance that develops in the environment may be clinically relevant across all sectors.

Many studies have documented antimicrobial residues in ecosystems influenced by both urban and agricultural activities. Likewise, ARGs and bacteria (notably zoonotic organisms) with resistance to one or more antimicrobials can be detected in surface waters, in soils, in animal feeds and on edible plants around the globe. In some regions, guidelines and regulations have been introduced to limit environmental contamination by industrial, human and animal wastes. However, there are still many gaps in knowledge about the ecology of AMR when it comes to environmental contamination with antimicrobial residues, resistant bacteria, and ARGs. For example, the magnitude of the public health threat posed by antimicrobial-resistant organisms and ARGs in the environment, and the effects of antimicrobial residues on soil ecosystem services, such as biogeochemical cycles, are still unknown.

There are multiple potential sources of antimicrobials entering the environment. Among the most important anthropogenic contributors to environmental pollution with antimicrobials are wastes from pharmaceutical manufacturing, hospitals, wastewater treatment facilities, untreated human wastes, waste and runoff from aquaculture, livestock, and plant-based food production and processing facilities. However, the attributable fraction of each source, and factors governing abundance and distribution of antimicrobial-resistant organisms, ARGs, and residues in the environment from each source is unclear. Despite current knowledge gaps, there are several practical and immediate actions that can be taken to minimize environmental contamination with antimicrobial residues, antimicrobial-resistant organisms, and ARGs.

- With respect to agricultural sources, reducing the need for AMU through improved animal health and hygiene practices is the single most effective way to proactively reduce the contamination of animal wastes with antimicrobial residues and AMR bacteria. Biosecurity, vaccination, no stress and good feed at the herd or flock level is widely encouraged for many reasons and can be effective for preventing exposure of the herd or flock to antimicrobial-resistant organisms. Actions that target the transmission of specific animal diseases, particularly those caused by bacterial pathogens, are likely to be effective for preventing AMR entry in to the herd/flock.
- Animals may be colonized with antimicrobial-resistant organisms and this can result in a number of environmental niches being contaminated. Where animal production is itself a source of AMR for other food producing systems (including crop enterprises on the same farm), the biosecurity program must include strategies for containing AMR. This could include consideration of treatment of effluents, restricting the use of farm wastes, and ensuring commodities leaving the farm are sterile. Given the above, mangers should assess the risks related to AMR exposure, release and transmission through the environment and integrate appropriate mitigation steps into biosecurity practices.
- Waste treatment protocols vary in their efficacy to remove or decrease antimicrobial residues depending on the treatment process and the specific antimicrobial in question. Thus existing waste treatment protocols should be reviewed to assess their efficacy against AMR and adjusted as indicated by the findings. In low- and middle-income countries the waste treatment facilities and standard operating procedures may be limited in number or absent. In these circumstances awareness of the importance of AMR to human health, animal health and food production needs to be generated so that resources, infrastructure, and effective regulatory action can be directed at the issue.
- Protecting water from contamination with residues is the first step in reducing their impact on the environment. This may be achieved by regulating and enforcing the amount of antimicrobial residues discharged into the environment.

While many developed countries are aware of and practice various levels of environmental protection, more widespread and rigorous implementation of these practices specifically aimed at reducing antimicrobial residue pollution in the environment will contribute towards slowing the development of AMR – a priority for all countries in the context of the Global Action Plan on antimicrobial resistance.

Additional information, tools, and activities are urgently needed to better characterize and mitigate the risks associated with antimicrobial residues and antimicrobial-resistant bacteria from agronomic sources in the environment, particularly in LMICs. Paramount among research priorities is determining the magnitude of the direct and indirect public health costs posed by environmental contamination with antimicrobial residues, antimicrobial-resistant organisms, and ARGs. Of comparable urgency is the need to determine the relative fraction of contamination attributable to the various potential sources of environmental antimicrobial residues so that interventions and resource allocation can be prioritized for maximum impact and return on investment.

Additional information is also needed to better understand the impact, effectiveness, costs and benefits of different waste treatment practices, such as composting and manure storage, biochar formation, anaerobic digestion, ozone and ultra-violet light treatment, among others. The role of wildlife as reservoirs in disseminating AMR and ARGs across sectors warrants study as well. Key variables to measure in future studies include: the interactive effects of the environmental matrix and conditions; the specific microorganisms of interest for the antimicrobial under study; persistence of antimicrobial residues, antimicrobial-resistant organisms, ARGs; and the impact of soil resistome (*i.e.* collection of ARGs) composition on ecosystem services in general. The latter may be especially relevant for food productivity and safety.

2.1 Soil

Livestock and humans that have received antimicrobials excrete active antimicrobial residues and bacteria carrying ARGs (Liu *et al.*, 2016; Pope *et al.*, 2009; Zhu *et al.*, 2013). Thus, manure or other organic material that contains human or animal wastes used as soil amendments, as practiced worldwide, have the potential to disseminate both residues of antimicrobial agents and antimicrobial-resistant bacteria into the environment (Jechalke *et al.*, 2013; Marti *et al.*, 2013; Marti *et al.*, 2014; Muurinen *et al.*, 2017; Pourcher *et al.*, 2014; Rahube *et al.*, 2014; Zhou *et al.*, 2017a; Joy *et al.*, 2013; Xie *et al*, 2018). The fate of these antimicrobial-resistant bacteria, ARGs and antimicrobial residues following application of soil amendments will vary with environmental conditions. For example, the selective properties of the antimicrobial residues can last for weeks to months, and possibly more than a single growing season in humid-temperate regions (Marti *et al.*, 2014; Chen *et al.*, 2018).

2.2 Irrigation water

Water can also be an important source of antimicrobial residues, antimicrobial-resistant bacteria and ARGs, if the irrigation water (or the soil) comes in contact with the plant part that is consumed (Palacios *et al.*, 2017; Pan and Chu, 2018). There is a direct link between water quality used for irrigation and antimicrobial-resistant bacteria on foods. Wastewater effluent recovered from municipal sewage may contain ARGs and antimicrobial-resistant bacteria (Berendonk *et al.*, 2015; Christou *et al.*, 2017; Karkman *et al.*, 2018; LaPara *et al.*, 2011) and may contaminate irrigation water. Water found adjacent to manured fields may also be enriched in antimicrobial-resistant bacteria (Coleman *et al.*, 2013; Pruden *et al.*, 2006). Comparison of fresh produce and its agricultural environment indicated that the Enterobacteriaceae population on fresh produce is a reflection of that present in the soil in which it was grown (Blaak *et al.*, 2015). A high degree of genetic relatedness between *E.coli* from irrigation water and lettuce indicated a possible common waterborne pathway of transmission (Aijuka *et al.*, 2015; Njage and Buys, 2015).

In a Brazilian study, forage maize and tanner grass irrigated with treated wastewater presented high levels of surface contamination with *E. coli* and *Salmonella spp.* (Bevilacqua *et al.*, 2014). Antimicrobial-resistant *E. coli* present in irrigation water and vegetables from 16 household farms were evaluated (Araujo *et al.*, 2017). The same sequence types and indistinguishable clones, as shown by rep-PCR typing, were detected in water and vegetables, suggesting cross-contamination. In a national soil survey, Northeast China was found to be a "hotspot" of ARGs, likely due to long-term wastewater irrigation in the area (Zhou *et al.*, 2017b). The presence of *E. coli* isolates from irrigation water and leafy green vegetables in different food production systems, such as large commercial farms, small-scale farms, and homestead gardens, was investigated (Jongman and Korsten, 2016). In that study, the prevalence of multidrug-resistant *E. coli* was lower in isolates from farms certified as implementing specific good agriculture practices to prevent contamination (Global GAP-certified) than among isolates from noncertified commercial and small-scale farms and homestead gardens. An *E. coli* transmission link between the irrigation water sources and leafy green vegetables was established using both phenotypic (AMR) and genotypic (DNA fingerprinting) analyses.

Constructed wetlands are used as biological treatment of animal, human and industrial waste. The efficiency with regard to the removal of antibiotics and ARGs varies according to type of antibiotic and ARG (Chen *et al.*, 2016). In some locales, however, such wetlands are concomitantly used for food production (i.e. crops and/or food of aquatic origin). New evidence indicates that these integrated wetland food production systems may be implicated in AMR spread (Krzeminski *et al.*, 2019).

2.3 Aquaculture

In 2014, with production of 73.7 million tons of aquatic animals (including marine and freshwater finfish, crustaceans and shellfish) with a value of 130 billion US dollars, the contribution of aquaculture to supply food for human consumption overtook that for wild-caught fish for the first time (FAO, 2016a). In 2016, this value increased to 80.0 million tons (FAO, 2018). Diseases are still considered to be a major constraint to aquaculture globally. It has been estimated that 10% of all cultured aquatic animals are lost because of infectious diseases alone, amounting to more than 10 billion US dollars in losses annually on a global scale (Evensen, 2016). Antimicrobials are routinely used in aquaculture for the treatment of bacterial diseases (FAO, 2016b). Although antimicrobials are effective in aquaculture, there are concerns over AMU (abuse, overuse, misuse) with regards to human, animal, and ecosystem health issues related to the development and dissemination of antimicrobial-resistant organisms through the environment (FAO/OIE/WHO, 2006).

Aquaculture products (*e.g.* fish, shellfish, and shrimp) at retail can carry bacteria that are resistant to medically important antimicrobials (Elbashir *et al.*, 2018; Done et al, 2015). Data indicate that aquaculture primary food production systems that receive antimicrobials, or that are exposed to effluents containing antimicrobial residues or faecal material of human or animal origin, can become enriched in antimicrobial-resistant bacteria (Novais *et al.*, 2018). Additionally, aquaculture production has the potential to contaminate water used downstream for irrigation (Done et al, 2015). Using water contaminated with this effluent for irrigation purposes provides a direct route of contamination of fruits and vegetables, if such water is applied directly to the edible portions of the plant (Watts *et al.*, 2017; Cabello *et al.*, 2013; Tendencia *et al.*, 2001; Miranda and Zemelman, 2002; Jang *et al.*, 2018).

Diversity of production systems (intensification, size, location, species, marine vs freshwater, *etc.*) needs to be considered when assessing the risk of AMR. Differences between aquaculture systems are remarkable among countries and may variably impact the risk of acquiring and disseminating AMR (FAO/WHO, 2003).The pathways for introduction and transmission of antimicrobial-resistant bacteria in aquaculture production systems include:

- Integrated food animal productions systems (*e.g.* poultry and fish) are common in small-scale aquaculture. There is evidence for development of AMR in these systems (Petersen *et al.*, 2002; Cabello *et al.*, 2016).
- Manure from swine and poultry production systems is used as feed or feed supplement in some aquaculture production systems (*e.g.* pond-raised tilapia) (Minich *et al.*, 2018; Elsaidy *et al.*, 2015).
- Run-off water potentially contaminated with human or animal waste can be directed into some fish ponds in some parts of the world to maintain water levels.
- There is a potential risk of foods of aquatic origin to be contaminated with AMR organisms both pre-harvest (water, sediment, effluent from the farms) and post-harvest (processing, packaging, storage) (Singh *et al.*, 2016).
- Sediment from retention ponds (*i.e.* lagoons) from some aquaculture production systems (*e.g.* freshwater rainbow trout in Chile) is used as fertilizer in horticulture (*e.g.* berry production).

- Antimicrobial-resistant organisms are common in the aquaculture environment, but there is a need for robust scientific evidence to assess the association between AMU and increase prevalence of antimicrobial-resistant organisms in the environment, in foods of aquatic animal origin, and their potential human health implications. It is also important to understand secular and seasonal trends in AMR in the aquaculture environment. More evidence about the pathways of AMR dissemination from aquaculture farms is needed as there is potential risk entering the food chain (For example, sediment used as fertilizer; Wellington *et al.*, 2013; Singer *et al.*, 2016; Bueno *et al.*, 2018). Collection of data on antimicrobial usage in aquaculture globally is needed. Standardised methods for measuring AMR in aquatic species pathogens is needed (Stärk *et al.*, 2018; Ngo *et al.*, 2018; Adams *et al.*, 2011). Co-selection for resistance may occur, but the extent that this contributes to AMR in aquatic animal production environments needs further investigation (Fernández-Alarcón *et al.*, 2010; He *et al.*, 2017).

2.4 Use of antimicrobials and copper in horticulture production

Antimicrobials, including gentamicin, streptomycin, kasugamycin, oxytetracycline, and oxolinic acid are vital to treat and control plant diseases (de León *et al.*, 2008; Stockwell and Duffy, 2012). Of these, streptomycin and oxytetracycline are approved for horticultural use in several countries. In New Zealand and the United States, antimicrobials (namely, streptomycin, oxytetracycline, kasugamycin) are mostly used for the management of fire blight disease of apple and pear, with use strictly regulated according to the chemical label. In these situations, antimicrobial applications are typically limited to bloom, approximately 4-5 months prior to harvest. However, in many other countries, AMU to treat plant disease is unregulated and unmonitored. The identity of the crop, disease targeted, spray timings, rates, frequency, and time to harvest are unknown.

Contamination of soils with these products following crop application leads to enrichment of antimicrobial-resistant bacteria and ARGs in the environment (Singer *et al.*, 2016). However, the extent to which the treatment of crops with antimicrobial agents (or copper formulations, see below) promotes AMR in bacteria found on edible portions of fresh plant produce is uncertain (Thanner *et al.*, 2016). While use information including total amounts used and crops treated is available from countries such as New Zealand and the United States, such information is not readily available from most countries (McManus, *et al.*, 2002; MPI, 2016).

Widespread use of streptomycin in horticulture, which began in many countries in the 1950's, was followed by the detection of streptomycin resistance in target plant pathogens including *Erwinia amylovora*, *Pseudomonas syringae*, and *Xanthomonas campestris* as early as the 1970's. This resistance is mediated by chromosomal mutations and plasmid-encoded genes. The most commonly described mechanism of streptomycin resistance is the acquisition of the *strAB* genes, which in many cases are located on Tn*5393* (Sundin and Wang, 2018). Despite their widespread use, resistance to kasugamycin, oxytetracycline and oxolinic acid in plant pathogens is less frequent (Sundin and Wang, 2018). It can be difficult to conclude that AMU is enriching the environmental reservoir of resistance because genes responsible for tetracycline and aminoglycoside resistance are naturally detected in bacteria isolated from soil and would likely be detected in most terrestrial habitats (Agga *et al.*, 2015; Versluis *et al.*, 2015). In some cases, screening of non-target bacteria isolated from orchards sprayed with streptomycin revealed the presence of the *strAB* genes and, in some cases, Tn*5393* (Norelli *et al.*, 1991; Sobiczewski *et al.*, 1991; Chiou and Jones, 1995; Sundin *et al.*, 1995). In addition, several tetracycline-resistance genes, including *tetA*, *tetB*, *tetc.*, and *tetG*, were present in tetracycline-resistant epiphytic bacteria in two apple orchards in Michigan state in the United States with no or limited exposure to oxytetracycline (Schnabel and Jones, 1999); however, tetracycline resistance has yet to be reported in the *E. amylovora* apple pathogen. Importantly, it should be noted that surveillance for AMR among phytopathogens is not global in scope, nor are samples often collected at time of harvest when the risks for food contamination would be most significant.

In contrast to AMU, copper-based bactericides are very commonly used (overall several orders of magnitude above antimicrobials) on a wider variety of crops, likely in most countries of the world as these compounds represent the sole bactericide available on many crops. Copper use also typically involves multiple spray applications per season. Copper resistance is widespread in plant pathogenic bacteria isolated from many continents and typically is plasmid-encoded (Lamichhane *et al.*, 2018). Of particular concern is the possibility of selection of antimicrobial-resistant bacteria and ARGs through the processes of co-resistance, cross-resistance and co-regulation with certain metal ions (Yu *et al.*, 2017). Evidence indicates that contamination of soil with certain metal ions, such as copper ions, promotes AMR in soil bacteria. Not only are copper-containing products used to treat plant diseases, animal and human wastes often have residue levels of copper, zinc and other metals of dietary or industrial origin. Pal *et al.*, (2015) conducted an extensive analysis of the associations between antimicrobial and metal resistance genes. Using a pollution induced tolerance approach, soils historically (>80 years) contaminated with copper were found to have an increased level of resistance to antimicrobials (*e.g.* vancomycin) implying that copper represents a strong pressure for co-selection of AMR (Berg *et al.*, 2010).

Co-resistance can occur when the genes for resistance to antimicrobials and metals are both present in a bacterium, such as found in approximately 5% of bacterial isolates recovered from plants and soils (Pal *et al.*, 2015). Bacteria harbouring genes conferring resistance to certain metal ions, and in some cases to certain biocides, are more likely to also encode ARGs than those without such metal or biocide resistance traits (Pal *et al.*, 2015). Bacteria resistant to both metal ions and antimicrobials are commonly present in diverse environments, with bacteria of plant origin having the highest relative abundance of co-resistance genes per genome, compared to bacteria from other sources such as domestic animals or wild animals and humans (Pal *et al.*, 2015). In the aforementioned study by Pal *et al.*, none of the bacterial isolates of plant or soil origin analyzed harboured genes for AMR and metal tolerance on the same plasmid, thus suggesting a limited significance of co-selection by metal for the horizontal gene transfer of antimicrobial resistance plasmids.

Nevertheless, plasmids co-encoding for metal and AMR have been identified in bacteria isolated from humans and animals (Bennett, 2008; Argudín *et al.*, 2017). Feeding of copper sulfate to pigs, as is widely practiced for health and growth promotion purposes.

Doing so however may select for *Enterococcus faecium* populations resistant to macrolides and glycopeptides (Hasman *et al.*, 2006). For example, in *E. faecium*, copper resistance (*tcr*), macrolide resistance *erm*(B) and glycopeptide resistance (*vanA*) are all encoded on a common plasmid. In other studies with Enterococci, co-transfer of copper tolerance (associated with *tcr*B, *cue*O, or an unknown mechanism) and erythromycin, tetracycline, vancomycin, aminoglycosides or ampicillin resistance was demonstrated indicating genetic linkage between copper tolerance and resistance to several classes of antimicrobials (Silveira *et al.*, 2014). In another example involving a foodborne pathogen, an emerging clinically important clone of *Salmonella enterica* 4,[5],12:i:- with co-resistance to copper and multiple antimicrobials, is circulating in Spain and Southern Europe (Mourão *et al.*, 2015), a region where copper is used extensively in both horticulture and animal agriculture. Copper is known to co-select ARGs including *ermB* and *vanA*, during use in animal agriculture (Seiler and Berendonk, 2012; Pool, 2017). Finally, in one example from plant production, co-resistance to both copper and streptomycin was identified in *P. syringae* strains exposed to both compounds (Sundin *et al.*, 1993). Since manure and sludge are used in horticulture as a fertilizer, the use of copper for plant protection could select for AMR among enteric bacteria present in these soil amendments.

2.5 Defining environmental quality thresholds for antimicrobials

2.5.1 Minimal inhibitory concentrations

Management targets related to the crop and environment would ideally be set at concentrations that are below the lowest concentration that allows antimicrobials to select for ARGs. Currently, the best estimate of where this might lie for each antimicrobial is based on experimentally-defined and modelled values (Andersson and Hughes 2012, 2014; Bengtsson-Palme and Larsson 2016; Gullberg *et al.* 2011, 2014; Hughes and Andersson 2012; Khan *et al.* 2017; Kraupner *et al.* 2018; Liu *et al.* 2011; Mezger *et al.* 2015; Murray *et al.* 2018; Sandegren 2014; Strukova *et al.* 2016). These experimentally-determined estimates of these thresholds are called minimum selective concentrations (MSC). The MSC is the lowest concentration of antimicrobial at which resistance is positively selected, whereas the minimal inhibitory concentration (MIC), a term commonly used in describing the lowest concentration of antimicrobial at which cell growth is visibly inhibited. The MSC can be significantly lower than the MIC (Sandegren 2014). Modelled estimates of an MSC can be found in Bengtsson-Palme and Larsson (2016). The authors derive MSCs from species sensitivity distributions populated with data from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) database. The authors selected the concentration of each antimicrobial representing the 1% potentially effective fraction, upon which a safety factor of 10 was added to account for the observation that experimentally-derived thresholds tend to be approximately an order of magnitude lower than the MIC, while also offering an added level of protection to the estimate. The 111 antimicrobials thresholds ranged from 0.008 µg/L to 64 µg/L. Among the more persistent and ubiquitous classes of antimicrobials are the macrolides and fluoroquinolones. The modelled MSC for these can be found in Table 2.

Table 2. Example modelled minimum selection concentrations for macrolides and quinolones (Bengtsson-Palme and Larsson, 2016)

Antimicrobial class	Drug	Bengtsson-Palme and Larsson (2016)		AMR-Alliance (2018)
		Modelled (µg/L)	Observed Lowest MIC (µg/L) in database	PNEC-env (µg/L)
Quinolones	Ciprofloxacin	0.064	2	0.45
	Levofloxacin	0.25	4	Testing ongoing
	Moxifloxacin	0.125	2	N/A
	Norfloxacin	0.5	16	120
	Ofloxacin	0.5	8	10
	Azithromycin	0.25	16	0.02

Macrolides	Clarithromycin	0.25	8	0.08
	Erythromycin	1	16	0.50

The evidence-base for establishing MSC targets for mitigation is currently poor. The reliability, if the MSCs obtained from competition experiments, is likely to be limited when extended to more complex microbial communities (Bengtsson-Palme *et al.*, 2014). The body of research needed to inform such targets will likely take a long time to accumulate. In the interim, it is prudent to use modelled MSC estimates.

2.5.2 Co-selection

When predicting drivers for resistance emergence under environmental conditions, it is important to account for the co-selection mechanisms, such as co-resistance and cross-resistance (Baker-Austin *et al.*, 2006; Di Cesare *et al.*, 2016; Pal *et al.*, 2015; Seiler and Berendonk, 2012; Zhao *et al.*, 2017). Co-resistance refers to different resistance genes present on the same genetic element (*e.g.*, plasmid, transposon, integron), while cross-resistance refers to the same gene conferring resistance to multiple chemicals (*e.g.*, multi-drug efflux pumps), which can be enriched for by a wide range of antimicrobials, biocides and metals. Unfortunately, there is currently no mechanism for estimating the impact from complex mixtures of co-selecting pollutants present in the environment on AMR selection thresholds. However, it is widely assumed that the threshold estimates are not likely to increase, but might further decline (Gullberg *et al.*, 2014). The concept of MSC should equally apply to biocides and metals, however, no published data currently exists.

3. Biocides in food production and AMR

Biocides, notably those used for premise and equipment disinfection and sanitation, are of critical importance for food safety to control microbial cross-contamination and ensure general hygiene at many stages of the food value chain. Active ingredients in biocidal products include a diverse collection of chemicals that may exert a microbiocidal or microbiostatic impact through a range of different mechanisms targeting a broad spectrum of microorganisms. Tolerance to biocidal products may be mediated through transient changes in genotypes and phenotypes (*e.g.* upregulation of endogenous genes, mutations). Some individual stressors may also co-select for resistance to multiple classes of antimicrobial drugs because of shared resistance mechanisms (cross-resistance) or genetic linkages (co-resistance) among resistance genes. If cross-resistance to antimicrobials or co-selection of ARGs is driven by routine biocide use, these unintended consequences need to be evaluated and considered by relevant stakeholders (*i.e.*, manufacturers and users) and appropriately managed (Webber *et al.*, 2015).

Currently, biocide use is being questioned due to the possibility that exposure could select for resistance to different antimicrobials and the induction of lateral gene transfer (LGT) (Wales and Davies, 2015). Moreover, the presence of biocide residues in the food production environment where antimicrobials are used can facilitate horizontal gene transfer (HGT) (Verraes *et al.*, 2013). There is ample theoretical and experimental evidence that certain biocide agents may co-select for AMR. For example, increased tolerance to biocide compounds in a few bacterial species of relevance in health and food processing environments has been recently documented (Hardy *et al.*, 2018). Laboratory experiments have demonstrated that interaction between biocides and antimicrobials can influence the development of either tolerance/resistance or collateral sensitivity to different compounds (Curiao *et al*, 2016; Peter *et al.*, 2018; Oxaran *et al.*, 2018).

However, evidence that biocides select for AMR is based on *in vitro* experiments following guidelines designed for testing antimicrobial susceptibility using planktonic bacteria (not biofilms) exposed to biocides in aqueous solution either with or without the addition of other co-solvents as ethanol or DMSO-conditions that do not mimic the natural environment (Bas *et al.*, 2017; CLSI, 2015). Examples include the use of chlorhexidine resulting in colistin resistance, or triclosan inducing isoniazid resistance. Only a few relevant studies reported some data about the co-tolerance (Romero *et al.*, 2017), and more data is needed to address the real situation in the field.

One factor potentially contributing to the development of resistance between biocides, and potentially cross-resistance to antimicrobials, is the use of biocidal agents that rely on a narrow mode of action (*i.e.*, acting on only one or a few bacterial targets). By contrast, resistance to biocides acting on multiple bacterial metabolic pathways would require the simultaneous acquisition of multiple resistance mechanisms to permit the microorganism survival; a process that is less likely to occur than the acquisition of a single resistance mechanism. While biocides are critical tools for hygiene and food safety, they have the potential to co-select for AMR, therefore, stakeholders need to be made aware of this risk to have the opportunity to conduct risk assessments and implement appropriate strategies to minimize its occurrence.

As more information becomes available to fill research gaps, more specific practices to measure and mitigate the effects of biocide use on AMR development can be promoted. Further research is needed to characterize the potential risks associated with cross-resistance to antimicrobials due to biocide use. Priority data gaps to address include:

- Studies *in situ* under realistic conditions with biocide products and key reference microorganisms or relevant commensals. Biocides are normally formulated with surfactants, sequestrants, and other disparate compounds. The few available reports using formulation containing surfactants and sequestrants to base assessments of the risk of induction of changes in bacterial susceptibility are not conclusive (Forbes et al., 2016).
- Investigations of the origin of antimicrobial-resistant bacteria found in the food value chain to determine the fraction, if any, attributable to the use of biocides.
- Standardized methods to measure and monitor biocide resistance.

Despite current gaps in knowledge, immediate action can be taken to mitigate potential risks by providing clear guidance to manufacturers and users of biocidal products on practices aimed at minimizing the potential development of resistance. For instance, the appropriate use of biocides in keeping with the manufacturer's instructions and the intended product use, and validation of effectiveness specific to the application are important in slowing the development of resistance. Improper or excessive use of biocides in the entire food production chain should be avoided as it may potentiate the problem of AMR emergence. Examples of inappropriate use are dilution below the working concentration and using biocide products outside their intended and validated application area. Manufacturers should provide clear instructions to users in this regard and stakeholders would benefit from education and awareness campaigns on proper usage.

Some biocides leave residues that are subsequently discharged into waste streams or otherwise contaminate the environment where they could select for AMR. Therefore, disinfectants that remain active even when they are washed away from food products may be less appropriate for use as biocides in food production and processing because of this potential for widespread impact on the environment and emergence of resistance. Use of technologies to inactivate residual disinfectants, before introducing them into wastewater streams, may be beneficial (Verraes *et al.*, 2013; Barancheshme and Munir, 2017).

Manufacturers may contribute to minimizing the likelihood of AMR developing by careful selection of active agents and formulations that target multiple bacterial sites and modes of action less likely to confer cross-resistance. In designing new biocide products, it would be prudent for manufacturers to investigate whether cross-resistance to clinically important antimicrobials is likely to occur under conditions of prescribed use. Conversely, users of biocides could be empowered and enabled to consider monitoring the potential occurrence of cross-resistance in their operations and investigate, where possible, causal links to biocide use or other triggers.

4. Integrated surveillance of AMR and AMU in crops, aquaculture products, and their production environments

Given the potential for human exposure to antimicrobial-resistant bacteria via foods of plant origin (Sundin and Wang, 2018) and from aquaculture products (Elbashir *et al.*, 2018; Done *et al*, 2015), programs for AMU and AMR surveillance needs to incorporate these food commodities and production systems. As well as sampling the food items themselves, specimens can be also collected from the immediate production environment from which the edible products are derived (soils, irrigation water, aquaculture water and sediments). Surveillance programs should take into account regional specificities and circumstances when selecting suitable fruit or vegetable products, fish and crustacean species and environmental samples for inclusion in such programs (Matheu *et al.*, 2017; Dorado-Garcia *et al.*, 2018).

Programs and tools to systematically measure and record antimicrobial contamination and antimicrobial-resistant bacteria in the environment at national levels are virtually absent. Environmental AMR surveillance systems need to be integrated and harmonized with surveillance in the human, animal, and food-chain sectors to track the spread of antimicrobial residues, antimicrobial-resistant organisms and ARGs to better assess the risks and priority areas for intervention. A key challenge in this work will be determining an appropriate standard denominator when expressing the magnitude of changes in environmental contamination so that progress within and across countries can be monitored in kind.

4.1 Elements of AMR surveillance system

AMR surveillance on crop, aquaculture and relevant food-production environments would be intended to capture trends over time. It would measure resistance (expressed as percentage resistance of total tested) and enable a comparison between sectors (human; animal and now foods of plant origin and foods of aquatic origin). Data captured should also provide information on AMU, so as to enable the identification of public health risks, and drivers of resistance. Potential consideration should be given to the practical aspects of data collection. Among these would be the indicator bacteria for each food commodity and environmental sources. Where appropriate, the same bacterial indicator organisms should be used across multiple sample types. For example, although *E. coli* may serve as a suitable common indicator bacterium for antimicrobial-resistant bacteria in foods of animal origin, there is a need to identify additional robust indicators of antimicrobial-resistant bacteria in food of plant origin and the immediate crop production environment. Likewise, there are no universally accepted bacterial indicators of AMR in aquatic products.

AMR surveillance should use culturing and validated antimicrobial susceptibility testing methods. Standardized panels of antimicrobials have been published (WHO, 2017). Results should subsequently be reported as zone diameters or MICs and interpreted based on ECOFFs (Valsesia *et al.*, 2015). This basic protocol could be expanded to include methods that require more advanced technical complexity and resource requirements, for example molecular methods for ARG analysis, and antimicrobial residue chemical analyses and whole genome sequencing. AMR profiles are determined for cultured isolates. In addition, metagenomics analyses may provide better insight into the collection of ARGs that may circulate in the environment leading to new bacterial serotype- resistance genes combinations and be transmitted to humans through food consumption such as *E. coli* O104 on sprouts (King *et al.*, 2012; Frank *et al.*, 2011). Based on pilot studies and available data relevant to the commodity and the location, an appropriate sampling plan should be designed for AMR. Chemical residues or the active metabolites themselves should be surveilled, using established standard methods, with the consideration of sampling size and assistance of an epidemiologist and statistician, in place for foods of animal origin.

For the foods of plant origin commonly intended to be consumed by animals or by humans fresh or minimally processed, consideration should be given to the pathogens of importance to human health and where AMR may be a hazard. These could also include *E. coli* as a measure of hygienic production, *Salmonella* species as an indicator of animal- or human-derived contamination, and *Listeria* species as an environmental indicator of pathogenic *Listeria monocytogenes*, *etc*. For the foods of plant origin commonly intended to be consumed after cooking, the organisms to be assessed included *E. coli* as a measure of hygienic production, *Salmonella* species as an indicator of human and animal-derived contamination, and possibly *Pseudomonas* species as an common environmental bacterial contaminant as sentinel for AMR.

The surveillance should be tailored to the local agricultural practices since the production systems and food consumption patterns are different in different parts of the world. It should be noted that manure and contaminated water are important vehicles for transferring antimicrobial-resistant bacteria, ARGs, and antimicrobial residues from one production system to another, and into the food chain. Knowledge of how animals are managed, housed, fed and how their waste is handled in different regions of the world is critical in designing robust AMR surveillance systems that are able to capture points of entry of antimicrobial-resistant bacteria into the food chain. Specimens for consideration to augment and complement activities in terrestrial food animal surveillance include: manure solid/liquid used as a fertilizer, and samples from human sewage that is used as a fertilizer, in some regions. Finally specific food categories, soils and agricultural water can be included based on their probability of contamination and likelihood of being consumed raw or uncooked (Figure 1, solely as an example).

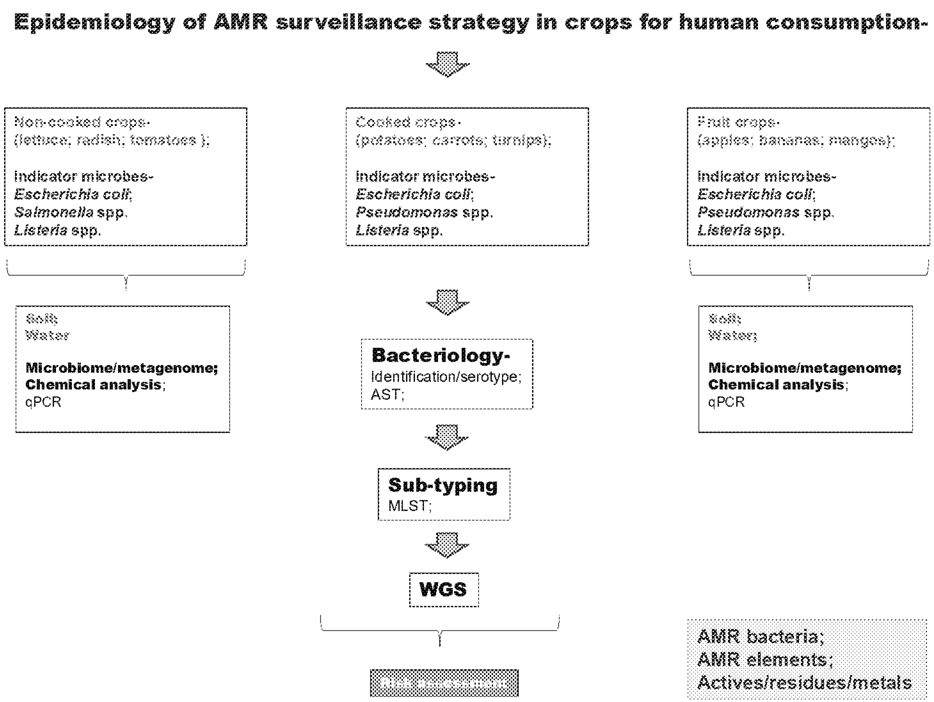

Figure 1 Schematic of AMR surveillance strategy in crops for human consumption

Antimicrobial-resistant bacteria, ARG and AMU surveillance in fruit and vegetable production systems should capture all important metadata for the antimicrobials such as information from manufacturers, importers and vendors, where possible. Analysis and reporting of these data should be performed on a quarterly basis. If possible, resistance data should also be compared with similar data, coming from human and animal surveillance platforms. Consideration to provide real-time alerts, such as through regional or international networks (*e.g.,* Rapid Alert System for Food and Feed (RASFF) in Europe, International Food Safety Authorities Network (INFOSAN)), might be taken, in the event of a detection of significant threat to public health (*e.g.*, a carbapenemase-producing bacterium) (Florez-Cuadrado *et al.*, 2018).

4.2 Antimicrobial use surveillance for crop and aquaculture

Collection of AMU information is an important component of an integrated AMR surveillance program (WHO, 2017). Representative, population-based AMU data provides information on the patterns and quantities of antimicrobial compounds that are being used in the country or region (European Medicines Agency, 2013). This information is useful for informing AMU policy and in the interpretation of AMR trends. Reducing AMU would be a key indicator to monitor progress of stewardship initiatives at global, country and local levels. Reducing inappropriate use will likely reduce selection pressure and therefore AMR. For example, in some countries AMU surveillance has been used to interpret AMR patterns in foodborne infections of humans (ECDC, EFSA and EMA, 2017), and to support efforts to reduce unnecessary AMU in food-producing animals (SDa Autoriteit Diergeneesmiddelen, 2016). WHO has published the guidance, in collaboration with FAO and OIE, on integrated surveillance of AMR including AMU surveillance as a key component (WHO, 2017), and OIE publishes a global annual report on the use of antimicrobial agents in animals (OIE, 2016). Currently, these documents focus on AMU in animal and human sectors, but do not specifically address horticulture, crops and aquaculture. There is a need to include these sectors in an integrated surveillance model to more comprehensively address AMR from a One Health perspective.

Effective AMU surveillance at the country level should aim to collect data from three sources, *i.e.* pharmaceutical manufacturers, at the point of sale and from the end user. This is recommended recognizing that a wide range of measures-ranging from policy initiatives to farm-level audits will be required to accomplish this goal. Targeting data from all three levels of the system permits triangulation complementation of information in the event data gaps.

Manufacturer-level data can be captured through mandating pharmaceutical companies to disclose information annually about the quantity of antimicrobials placed onto the market and for stated purposes and applications. Furthermore, these data should allow for the disaggregation of information to determine the sector that is purchasing the product (human, animal, crops), the classes of antimicrobials, whether for domestic or export, formations and bulk drugs. These data will extend the understanding of the potential selective pressures being imposed on these ecological niches.

Information acquired at the point of sale should include data on sales from importers, wholesale outlets and at retail level. Provisions should be made at the national and local level to ensure that all necessary documentation related to sales are collected and analyzed to understand sector specific sales segregated into type of antimicrobials.

Farm level usage data and prescription data (in the case of application to aquaculture; crops systems), although potentially difficult to acquire, are necessary to understand actual practices in regard to the use of antimicrobials. Provisions should be made to ensure farmers and prescribers maintain appropriate records and documentation to describe their antimicrobial compound usage data. These records should be available for audit by competent authorities.

5. Conclusions

5.1 Crop as a vehicle for AMR

- There is clear scientific evidence that foods of plant origin serve as vehicles of foodborne exposure to antimicrobial-resistant bacteria. As such, concerted efforts should be made to mitigate their contamination at all stages of the food chain, from production to consumption.

5.2 Food production environment

- In terrestrial food animal production systems, strict biosecurity should be introduced, including enhanced waste management. Risk assessments should be conducted to identify sources of environmental contamination. AMR surveillance programs in food-producing animals can be used to inform countries how to prioritize interventions (production systems and locations) that reduce the need for antimicrobials, thereby reducing the overall burden and transmission of antimicrobial-resistant organisms between animals, crops, and the environment.
- Improved methods for infection prevention and control such as husbandry, biosecurity, diagnostics, vaccines, standard methods, testing ideal and other alternatives should be employed to reduce the need for AMU in aquaculture. Consideration should be given to AMR surveillance in aquatic animal food production, in the animals and the pre- and post-harvest environments. Foods commonly consumed raw (*e.g.* raw fish, oysters, *etc*) should be ranked as highest priority amongst aquaculture products for inclusion in surveillance programs. Aquaculture sites should be positioned away from areas of sewage outflow.
- Best management practices should be adhered to with respect to the use of material of human (sewage sludge; biosolids) and animal (manures) origin in primary food production environments. Antimicrobials should only be used in crop production according to label guidelines in the context of integrated pest management strategies.
- Increasing awareness of the issue of antimicrobial residues and antimicrobial-resistant organism contamination in the environment is vital to drive changes in stakeholder practices. Requiring increased transparency on environmental aspects of waste management in food production, processing, and pharmaceutical production may further empower consumers to demand products produced by companies that prioritize environmental protection.
- A step-wise approach to antimicrobial stewardship in terrestrial animal food production, crop and aquaculture provides strategy for stakeholders to progressively implement changes to control AMR.

5.3 Biocides

- Presently, there is insufficient evidence to identify biocide use in food production as a driver of AMR. However, the identified associations between biocides tolerance and resistance to one or more classes of antimicrobials underscores the need for increased awareness and prudent use of these products.
- The wide range of biocide applications and targeted bacteria makes it difficult to establish relevant, standardized procedures for biocide susceptibility testing. Nevertheless, harmonized protocols are critically needed for biocide susceptibility testing. Recommendations for methods, ECOFFs, culture methods, biocide storage period according to the manufacturers' instructions, and control strains, should be developed.

- Monitoring the occurrence of biocide tolerance and cross- and co-resistance in the food production and processing environments should be undertaken. Such monitoring may complement on-going monitoring programs for hygiene and sanitation on AMR.

5.4 AMR and AMU surveillance

- Plant and aquatic animal food products and their production environment should be integrated into AMU and AMR surveillance program to support containment of AMR. The principles and methods used in existing WHO guidance and OIE report should form the basis of AMU surveillance in crops and aquaculture. It is recommended that AMR surveillance be implemented to capture potential seasonal and secular temporal trends. The prevalence of resistant organisms recovered from foods of plant and aquatic animal origin should be measured, by standardized laboratory methods, to enable a direct comparison between domains (human; animal; foods of plant origin & environmental sources) and facilitate identification of public health risks. Isolates of interest should be forwarded to a laboratory with sufficient capacity for confirmation (*e.g*. National Reference Laboratory) and publicly reported on a quarterly basis.
- Surveillance of AMU and antimicrobial-resistant bacteria in food commodities can provide an assessment of the magnitude of the problem and a tool for measuring progress in mitigation. At a local, regional and global scale there is insufficient knowledge about the amounts and types of antimicrobials applied to crops and those used in terrestrial agriculture and aquaculture. It is recommended that surveillance for AMR and AMU in primary food production environments be implemented in order to obtain additional data that is required for risk assessment and risk management. Terrestrial and aquatic primary food production system environments and products post-harvest should be considered for inclusion in integrated AMU and AMR surveillance programs foundational for containment of AMR.
- The development and enforcement of suitable regulatory instruments may be helpful to address potential misuse of antimicrobials, such as their application to products in the post-harvest period.
- A greater understanding of the role of food production environments in the transmission of foodborne antimicrobial-resistant bacteria and ARGs, and the role of agricultural use of antimicrobials and potential co-selective agents (*e.g.* copper ions, and potentially other antimicrobials) should incentivize the development of additional tools and strategies to reduce AMU and foodborne AMR.
- Generally, more education and training concerning AMU and AMR should be made available to all stakeholders involved with the use of antimicrobials in production of plant crops and aquaculture (FAO, 2017). To address upstream and downstream contamination of water and soils from human and animal faeces and antimicrobial-resistant organisms and ARGs in the environment, additional training and education on AMR and AMU in terrestrial and aquatic food production systems could also be beneficial.

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